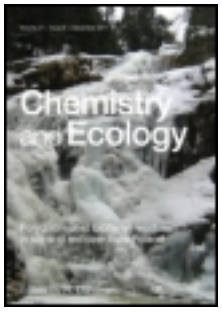

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Antifungal activity of volatile metabolites emitted by mycelial cultures of saprophytic fungi

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Antifungal activity of volatile metabolites emitted by mycelial cultures of saprophytic fungi

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The antifungal activity of molecular identified Chilean saprobiontic fungi, *Trichoderma viride, Schizophyllum commune* and *Trametes versicolor,* on the fungal plant pathogens *Botrytis cinerea* and *Fusarium oxysporum*, and the saprotrophic mould *Mucor miehei* was investigated using two types of inhibition bioassay: (1) bi-compartmented Petri dishes and (2) two Erlenmeyer flasks connected by their upper parts. The chemical composition of volatile organic compounds (VOCs) released by saprobiontic fungi was also investigated using headspace solid-phase microextraction (SPME) and gas chromatography/mass spectrometry (GC-MS) analysis. Of the saprobiontic fungi evaluated, one isolate of *S. commune* showed the highest inhibitory activity against *B. cinerea* and *M. miehei*, 86.0 ± 5.4 and $99.5 \pm 0.5\%$ respectively. The volatile profiles of fungal isolates were shown to contain a different class of compounds. The major components in the headspace of mycelial cultures were 6-pentyl-α-pyrone (*T. viride*), ethanol and β-bisabolol (*S. commune*), and a sesquiterpene alcohol (*Tr. versicolor*). This is the first study reported on the release of VOCs by Chilean native fungi and their antifungal activity *wrt.* plant pathogenic fungi.

Keywords: biological activity; VOCs; saprophytic fungi; phytopathogenic fungi; SPME

1. Introduction

Fungi produce, and release, volatile organic compounds (VOCs), including hydrocarbons, alcohols, ketones, aldehydes, ethers, esters, terpenes, terpene derivatives and several heteroaromatic compounds [1–3]. Fungal volatile organic compounds (FVOCs) have been detected from fungal fruit bodies [4], fungal-spoiled food [5] and fungi within buildings [6]. These compounds have been studied for a variety of purposes, including natural aromatic flavouring [7], pollinator attractants [8,9], indicators of fungal contamination [3] and as biocontrol agents (BCAs) of agricultural plant pests and diseases [10–13]. The use of volatile-producing fungi and/or their VOCs as BCAs for pathogen control constitutes a real alternative to the use of chemical products

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[14,15] that have caused an increase in genetic resistance to fungicides (e.g. *Botrytis cinerea*), toxin accumulation in foods and environmental contamination. Volatile natural products released by fungi are attractive candidates for use as novel agents for controlling agricultural pests [16]. However, data concerning the volatile metabolites emitted by fungi as control agents are limited, and it is necessary to continue research on volatile-producing fungi and their potential application in biological control strategies of fungal plant diseases.

Saprobiontic fungi are the most important and efficient microorganisms involved in natural wood decomposition, where they degrade and take up nutrients from dead organic matter by means of enzymatic process [17]. *Trichoderma* sp. is one of the most studied biocontrol agents due to the production of degrading enzymes, antibiotic non-volatile and volatile compounds, and mycoparasitism [18]. The world's native forests preserve fungal strains with valuable biological properties that are waiting to be discovered. Saprobiontic fungi play an important role in these ecosystems because of their production of enzymes and secondary metabolites involved in the decomposition of dead organic matter and their competition behaviours with other microorganisms to colonise a niche [19]. Because of these characteristics, saprobiontic fungi isolated from the forest have the potential to provide novel candidates with antifungal activities. To date, the study of antimicrobial activity of saprobiontic fungi in Chile has been focused on non-volatile secondary metabolites [20–23]. The aim of this study was to investigate the production of volatile metabolites emitted by saprobiontic fungi growing in the temperate native forest of the south of Chile and, for the first time with native Chilean isolates, evaluate their respective antibiotic effects on phytopathogenic fungi.

2. Material and methods

2.1. *Fungi collection and growth conditions*

Chilean native fungal isolates were collected between July and October 2008 from the native forest Rucamanque located in south–central Chile (latitude, 38°39′S; longitude, 72°35′W). The forest was composed mainly of roble (*Nothofagus obliqua*), olivillo (*Aextoxicon punctatum*), tepa (*Laureliopsis philippiana*), ulmo (*Eucryphia cordifolia*), laurel (*Laurelia sempervirens*), lingue (*Persea lingue*) and tineo (*Weinmannia trichosperma*) [24]. Four native saprobiontic fungal isolates were obtained from fruit bodies and dead wood samples, transferred to the Center of Chemical Ecology of Terrestrial and Aquatic Systems at the Universidad de La Frontera (Chile), and kept at 4 °C until the cultivation of the respective myceliam. Phytopathogenic fungi (*B. cinerea* and *Fusarium oxysporum*) strains from highbush blueberries (*Vaccinium corymbosum*) were obtained from the fungal collection of Instituto de Investigaciones Agropecuarias (INIA-Quillamapu, Chile) and *Mucor miehei*, included in the assays as a reference strain, was obtained from the fungal collection of the Universidad de Talca (UTAL, Chile). The three test strains were grown on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) at 25 °C in the dark for 7 days and stored in both Petri dishes sealed with Parafilm at 4 °C and glass tubes containing 5 mL of PDA submerged in sterilised paraffin oil at 4 °C.

2.2. *Identification of saprobiontic fungi*

Identification of the fungal isolates was carried out using two methodologies: (1) morphological characteristics and (2) molecular analysis.

Morphological characteristics were determined in the Laboratorio de Química de Productos Naturales (Universidad de Concepción, Chile).

Genomic DNA of fungal isolates was obtained from fresh mycelia culture. The samples were frozen in liquid nitrogen and powdered using a mortar and pestle. They were resuspended in TE buffer (10 mM Tris/Cl pH 8.0, 1 mM EDTA), and an equal volume of lysis buffer was added (2% SDS, 10 mM Tris/Cl, 1 mM EDTA pH 7.0). After incubation on ice for 15 min, the mixture was centrifuged at 3000 ***g*** for 10 min. The supernatant was subjected to phenol/chloroform extraction, followed by ethanol precipitation [25]. The resulting pellet was resuspended in TE buffer containing 100 $\mu g \cdot mL^{-1}$ RNase A (Fermentas®) and incubated at 37 °C for 30 min. The suspension was then subjected to phenol/chloroform extraction and ethanol precipitation. Whole genomic DNA was dissolved in deionised and nuclease-free water.

DNA samples were subjected to PCR using the technique developed by Mullis [26]. The primers used were: forward, ITS1 5′-TCCGTAGGTGAACCTGCGG-3′ and reverse, ITS4 5′-TCCTCCGCTTATTGATATGC-3′, as described by White et al. [27] and synthesised by Invitrogen, USA. The primers were used to amplify a fragment of rDNA, including the ribosomal RNA gene, partial sequence; internal transcribed spacer (ITS) 1, 5.8*S* ribosomal RNA gene, and ITS 2, complete sequence; and 28*S* ribosomal RNA gene, partial sequence.

PCR amplifications were performed in a total volume of 50 μL by mixing 15 ng of the template DNA with 20 μM of each primer, 25 μM of each dNTP, and 2.0 U of *Taq* DNA polymerase (recombinant) and 10 × *Taq* Buffer (100 mM Tris/Cl, pH 8.8 at 25 °C; 500 mM KCl; 0.8% v/v Nonidet-P40, Fermentas®). These reactions were subjected to an initial denaturation of 5 min at 95 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72°C, with a final extension of 5 min at 72 °C in a Labnet MultiGene™ 96-well Gradient Thermal Cycler. Aliquots (2 μL) were analysed by electrophoresis in 1.2% (w/v) agarose gel in 1× TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA pH 8), stained with ethidium bromide and photographed over a transilluminator. The molecular size marker was GeneRuler™ 100bp Plus DNA Ladder (Fermentas®) (not shown data). Results were obtained in Laboratory of Biology and Biochemistry Soil (Universidad de La Frontera, Temuco, Chile).

Fragments of the ribosomal DNA region, including the spacers ITS1 and ITS2, genes 5.8*S* (complete sequence), SSU and LSU (partial sequence) were sequenced in both directions using an automated DNA sequencing system (ABI, Model 3730XL Genetic Analyser, by Macrogen, Korea). Sequence was compared with Basic Local Alignment Search Tool (BLAST) data base.

2.3. *Antifungal activity*

Antifungal activity was evaluated by developing two methodologies: (1) two-compartmented plate bioassay, and (2) Erlenmeyer flasks connected by their upper parts.

2.3.1. *Two-compartmented plate bioassays*

Two-compartmented Petri dishes (94 × 16 mm; 47 mL) (Greiner, Germany) were filled with 50 mL 4% of PDA. One of the compartments was inoculated in the centre with a 6-mm diameter mycelium disc of fresh culture of test strain (target fungus), and the second compartment was inoculated with a disk of fungal study isolate of the same size. The control treatment was prepared by applying the test strain in one compartment and evaluating their mycelial growth without the presence of the fungal isolate in the other compartment. The preparations were placed together and wrapped around the outer surface with two layers of Parafilm M®, then incubated at 25 ± 1 °C, until the growth of any fungi (test and control treatments) reached the edge of the plate. The mycelial growth (colony diameter in mm) was measured with the help of an optical microscope (Japan Optical), and the data were expressed as percentage inhibition in relation to the control. In another experiment, test strains were exposed to 15 μL of 3-methylbutyl

acetate (99.9%, Sigma-Aldrich), added to 6-mm filter disks before the start of the experiment, and grown on PDA at 25 °C. This compound was selected as a positive control, because it has been reported as a significant inhibitor of the mycelium growth of phytopathogenic fungi [10]. The amount of 3-methylbutyl acetate was determined in relation to data reported by Strobel et al. [10]. A preliminary bioassay was assessed with the aim of determining the appropriate moment of the inoculation of each strain. In order to do this, study isolates and test strains were cultivated on Petri dishes with the same substrate, and the growth was daily measured daily to determine the time when each strain reached the edge and/or the central division of the Petri dish. The recorded times were used to calculate the moment of inoculation of each strain, with the purpose of avoiding the invasion of another compartment before the bioassay was ended. In this type of bioassay, each test strain was tested with all the study isolates. Each experiment was repeated at least four times.

2.3.2. *Erlenmeyer flasks*

A 250-mL Erlenmeyer flask, charged with 50 mL of 4% PDA, was inoculated in the centre with a 6-mm diameter mycelium disc of fresh culture of one the three most bioactive studies isolate, as determined in the two-compartmented plate bioassay. The cultures were covered with three layers of Parafilm M® and incubated at 25 ± 1 °C until growth reached the edge of the flask. At this stage, another Erlenmeyer flask containing 50 mL of 4% PDA was inoculated in the centre with the most inhibited plant pathogenic fungus, as determined in the two-compartment plate bioassay. Immediately, both flasks were joined by their upper parts and separated by a glass microfibre filter (Whatman, GF/D, 47 mm dia. and 2.7 μm in particle retention) placed in the centre to prevent the transfer of spores between species. The two Erlenmeyer flasks were coated with polytetrafluoroethylene (Teflon) tape where they were joined and incubated at 25 ± 1 °C until the growth of the control treatment reached the edge of the flasks. The control was the same bioassay without the addition of a culture of study isolate. Three replicates were developed for all tests and controls.

2.4. *Volatile compound collection by SPME*

Study isolates were cultivated in 40-mL SPME glass vials (Supelco, Inc., PA, USA) with 10 mL of 4% PDA and incubated for 10 days at 25 ± 1 °C in the dark. Volatile compounds were absorbed by headspace SPME (HS-SPME) for 10 h at 25 °C with a 100-μm polydimethylsiloxane (PDMS) fibre, as described previously by Chuankun et al. [28]. After each volatile trapping, the fibre was conditioned with helium for 10 min at 250 °C. Controls of SPME trapping consisted of sampling vials that contained PDA, maintained in the same conditions as for the treatments.

2.5. *Analysis of volatile compounds by GC-MS*

The volatile compounds collected by SPME were analysed using a gas chromatograph (Model Focus, Thermo Electron Corporation, Waltham, USA), coupled to mass spectrometer (Model DSQ, Thermo Electron Corporation), equipped with a DBP-1 capillary column (30 m, 0.2 mm, 0.33 μm). Helium was used as the gas carrier, with a flow rate of 1.5 mL · min^{-1}. Mass spectra were acquired in the mass range of 35–500 a.m.u. Ionisation was performed by electron impact at 70 eV with an ion source temperature set at 200 °C. The SPME fibre was inserted into the injector of the gas chromatograph for thermal desorption in splitless mode for 2 min, with the injector temperature held at 250 °C. The GC oven temperature was programmed to ramp from 40 to 260 °C at 5 °C · min^{-1} and then held for 5 min. Volatile compounds were tentatively identified by comparing: (1) mass spectra with data from the NIST MS Search 2.0 library, and (2) experimental

Kovats indices (KI) with theoretical KI values from synthetic standards compounds reported in the US National Institute of Standards and Technology (NIST) Mass Spectral Library database (NIST, http://webbook.nist.gov/chemistry/name-ser.html).

2.6. *Statistical analysis*

The results of both assays were expressed as the percentage inhibition of radial growth compared with the control. Data were averaged and the standard error of the means (SEM) was calculated. Inhibition percentage data were transformed using an angular transformation (arc sen $\sqrt{x/100}$) prior to statistical analysis. Post hoc analysis of differences in means of the assay data was conducted with the Tukey test ($\alpha = 0.05$) using the SPSS statistical program (SPSS for Windows version 10.0.1).

3. Results and discussion

3.1. *Fungi identification*

Morphological analysis of fungal collections revealed that two belonged to the genus *Schizophyllum*, one to *Trametes* and one to *Trichoderma*. The nucleotide sequence of ITS regions containing the 5.8*S* ribosomal gene were obtained from isolates of these four collections and compared using the BLAST. The maximum likehood tree test (Figure 1) based in sequence similiraties showed that two isoaltes grouped with *S. commune*, one with *T. viride* and one with *Tr. versicolor*. However, *S. commune* (Shi-1) and *Tr. versicolor* isolates differed genetically from previously known reference strains, suggesting that it may represent a regional ecotypes of these species (Figure 1a and d, respectively). Test strains obtained from culture collections were also molecurlarly characterised and their identitities with *B. cinerea*, *F. oxysporum* amd *M. miehei* confirmed.

In summary, the saprophtytic strains used in this study were two *S. commune* (JF828027 Shi-1; JF694037 Shi-2), *Tr. versicolor* (JF828026 Tra-1) and *T. viride* (JF828028 Tri-1). Plant pathogenic fungi were also molecular identified, isolates resembled *B. cinerea* Pers.:Fr. (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel), *F. oxysporum* and *M. miehei* (*Rhizomucor miehei*).

3.2. *Antifungal activity*

Two methods were used to evaluate the antifungal activity of volatile metabolites released from the study isolate on mycelial growth of test strains. The first approach comprised a bi-compartmented Petri dish array. The first bioassay, which was a relatively simple and rapid test modified from Strobel et al. [10] and Gu et al. [29], allows the evaluation of the inhibitory activity by volatiles emitted by microorganisms without interference from other compounds (e.g. enzymes and/or non-volatile metabolites). Using this assay showed that volatile metabolites of both *S. commune* isolates inhibited the growth of *B. cinerea* by only $\sim 20\%$ compared with the control treatments, whereas volatiles of *Tr. versicolor* and *T. viride* showed low antifungal activity. The positive control (15 μL of 3-methylbutylacetate) inhibited the growth of *B. cinerea*, *M. miehei* and *F. oxysporum* by $100 \pm 0.0\%$, $85.6 \pm 1.8\%$ and $66.6 \pm 0.8\%$, respectively (Figure 2).

Owing to the low percentage of inhibition observed using the bi-compartment Petri dish assay method, a second assay comprising two Erlenmeyer flasks connected by their upper parts was used. This bioassay was modified from Dal Bello et al. [30], who evaluated the effect of volatile metabolites of *Trichoderma hamatum* on soil-borne phytopathogenic fungi. Although this assay can be used to evaluate a larger biomass of fungus-producing volatiles without interference from other

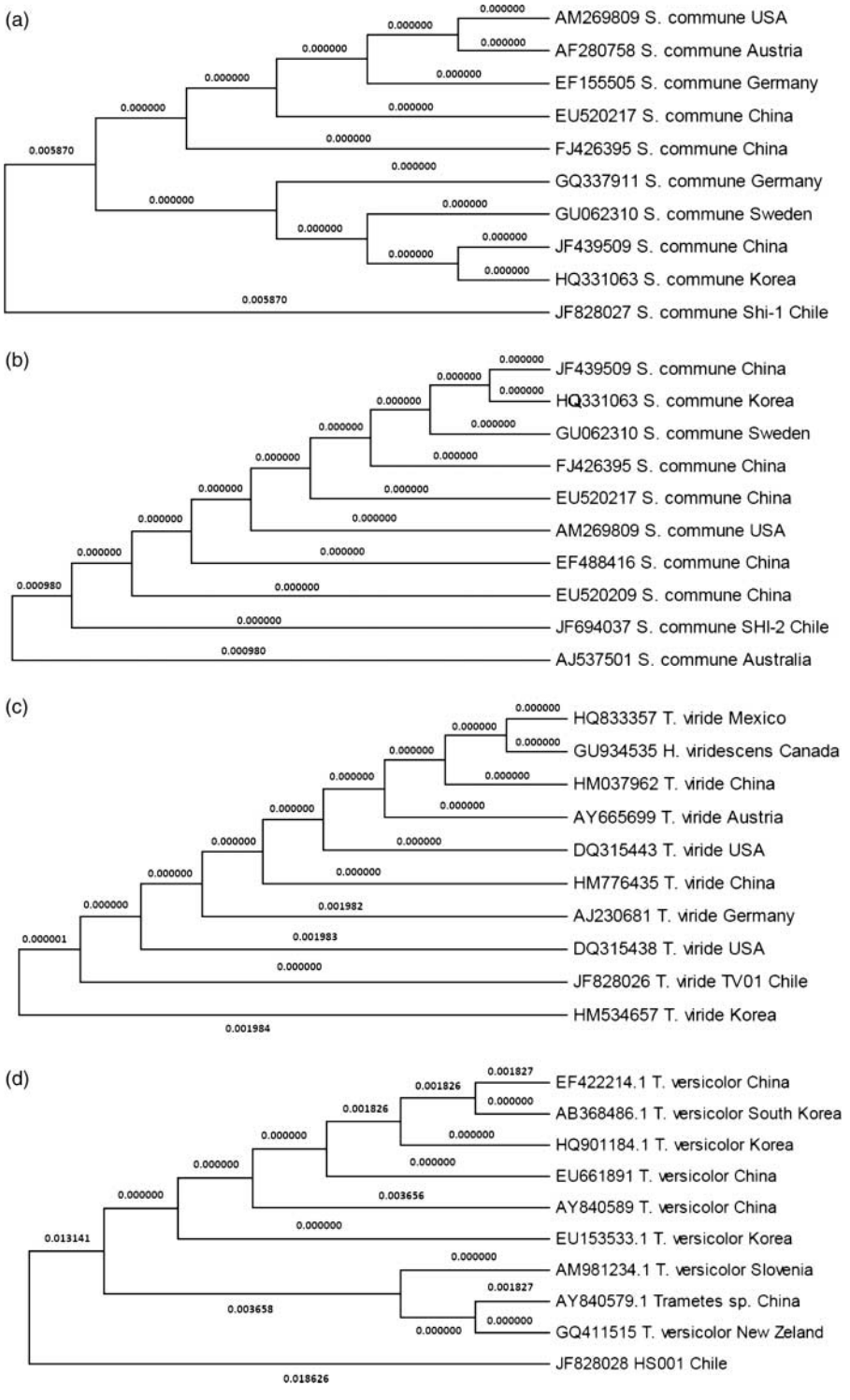

Figure 1. Dendrograms demonstrating the taxonomic relationship of the study isolates. (a) *Schizophyllum commune* (Shi-1), (b) *Schizophyllum commune* (Shi-2), (c) *Trichoderma viride* and (d) *Trametes versicolor*. The GenBank accession numbers of the sequences are given.

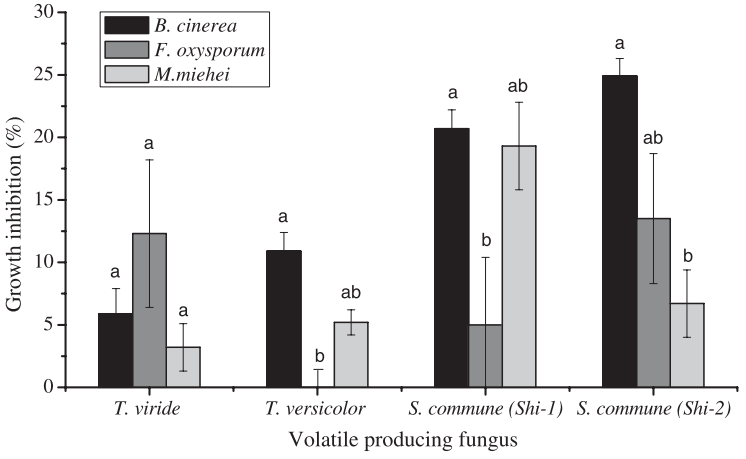

Figure 2. Mean growth inhibition (% ± SE) of *Botrytis cinerea*, *Fusarium oxysporum* and *Mucor miehei* exposed to VOCs emitted by four native saprophytic fungal isolates, grown on potato dextrose agar (PDA) at 25 ± 1 °C and evaluated using a bi-compartmented Petri dish assay ($N = 4$). Growth was expressed as percentage inhibition compared with the control (no volatile-producing fungus). Bars within a strain with the same letter are not significantly different based on the Tukey test ($\alpha = 0.05$).

metabolites, it has disadvantages in terms of the accumulation of carbon dioxide and decreased levels of oxygen. These experiments were carried out with only isolate/target strain combinations showing the highest inhibitory effect in the bi-compartment Petri dish bioassay. The isolates tested were: *S. commune* (Shi-2) with *B. cinerea* and *F. oxysporum*, and *S. commune* (Shi-1) with *M. miehei*. *S. commune* (Shi-1) inhibited the *M. miehei* growth by 99.5 ± 0.5% and *S. commune* (Shi-2) inhibited *Fusarium* sp. and *B. cinerea* growth by 63.3 ± 1.2% and 86.0 ± 5.4%, respectively (Figure 3). The increase in the inhibitory effect of *S. commune* strains using this method may be due to an increase in the mycelium biomass producing volatiles, because the flasks have a

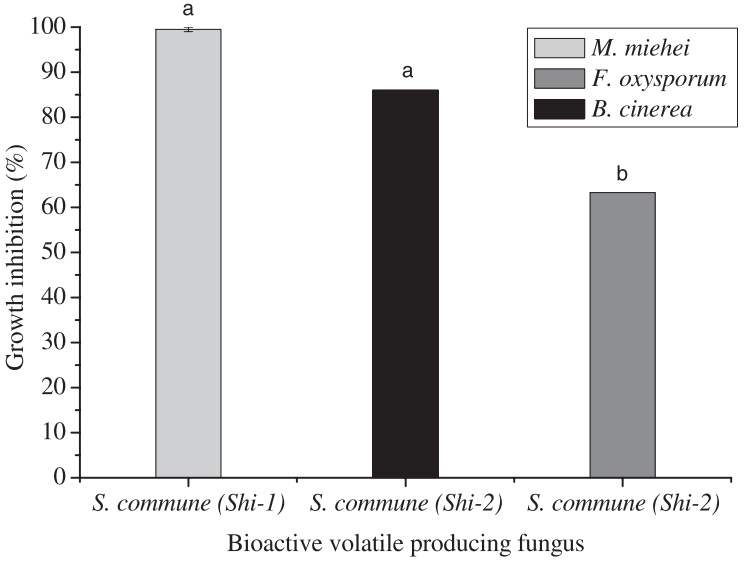

Figure 3. Mean growth inhibition (% ± SE) of test fungi exposed to native wood decay fungal isolates using a two Erlenmeyer flask assay ($N = 4$). Growth was expressed as percentage inhibition compared with the control (no saprobiontic fungus). Values of bars with different letters are significantly different based on the Tukey test ($\alpha = 0.05$).

greater surface area for mycelium growth than Petri dishes. Alternatively, growth of target fungi may be more sensitive to the high CO_2 and low O_2 levels in the closed flask system.

Very little data exists concerning the antifungal effect of volatile compounds emitted by saprobiontic fungi on plant pathogenic species. Most of the reported studies have focused on volatiles released from the biocontrol agent *Trichoderma* sp. Dal Bello et al. [30] carried out a similar assay to study the effect of volatile metabolites from the saprobiontic fungus *Trichoderma hamatum* on the growth of phytopathogenic soil-borne fungi using the same substrate (PDA) as in our study, and observed that the growth of three soil fungi strains were inhibited by >60%, two strains were not affected, and one strain was stimulated. Our results showed inhibition percentages >80% on *B. cinerea*, a plant pathogen that infects post-harvest berries and others crops in Chile and other parts of the world.

3.3. *Headspace analysis*

The profiles of the volatile compounds emitted by the native saprophytic isolates tested are shown in Table 1. The main components identified by GC-MS were 6-pentyl-α-pyrone (6-PAP) (*T. viride*), ethanol (*S. commune*, Shi-1), β-bisabolol (*S. commune*, Shi-2) and a sesquiterpene alcohol (*Tr. versicolor*). 6-PAP is a characteristic compound of the genus *Trichoderma*

Table 1. Chemical composition of volatiles obtained from mycelial cultures of saprophytic fungal strains using solid–phase microextraction (SPME).

		Native fungal strains (relative area, % ± SD)			
Compound	KI	*Trichoderma viride*	*Trametes versicolor*	*Schizophyllum commune* (Shi–1)	*Schizophyllum commune* (Shi–2)
Ethanol	–	–	–	59.19 ± 4.93	25.38 ± 3.31
Mercaptoacetone	–	–	–	–	6.96 ± 2.48
3-Methyl-1-butanol	–	–	9.52 ± 4.4	–	–
2-Methyl-1-butanol	–	–	3.53[b]	–	–
2-Methyl-1-propyl acetate[a]	758	–	–	–	2.36 ± 0.04
Ethyl 2-methylbutanoate	836	–	–	13.39 ± 0.37	2.32 ± 0.99
2-Methylbutyl acetate	863	–	–	10.04 ± 1.33	5.63 ± 0.90
6-Pentyl-α-pyrone	1434	96.71 ± 0.65	–	–	–
β-Himachalene[a]	1469	0.26 ± 0.03	–	–	1.73 ± 0.54
m/z = 93 (100), 121 (97), 39 (79), M^+ 164 (79), 107 (73), 79 (67), 95 (63), 77 (59), 110 (41), 123 (34), 55 (28), 136 (19)	1478	1.50 ± 0.26	–	–	–
α-Bergamotene	1482	–	–	2.71[b]	1.39 ± 1.05
β-Bisabolene[a]	1498	0.16 ± 0.04	–	6.96 ± 0.02	6.36 ± 1.48
Cadinene[a]	1497	–	11.29 ± 5.5	–	–
Spathulenol[a]	1553	–	18.67[b]	–	–
m/z = 203 (100), 218 (40), 147 (16), 69 (13)	1610	–	1.39 ± 0.7	–	–
β-Bisabolol[a]	1652	–	–	9.09 ± 1.34	35.67 ± 11.02
m/z = 137 (100), 81 (62), 95 (44), 69 (38), 41 (34)	1714	–	48.19 ± 10.6	–	–
m/z = 152 (100), 121 (92), 69 (70), 41 (47),	1736	–	–	–	5.56 ± 0.07
m/z = 69 (100), 121 (99), 166 (85), 138 (76), 41 (55)	1808	–	–	–	5.43 ± 0.01

Notes: Data are averages of two cultures grown on the same medium. Compounds appearing in the control PDA plate and with values <1% were omitted from the list of compounds. All volatile compounds were identified by comparing mass spectra with data from the NIST MS Search 2.0 library. [a]Compounds identified by comparison of experimental Kovats Index (KI) with literature data. [b]Compounds detected in only one repetition.

spp., and has caused great interest due to its strong coconut-like aroma [31], phytotoxic activity [32] and antifungal activity [11]. The antifungal activity of 6-PAP, synthesised artificially and/or by different strains of *Trichoderma* spp., on plant pathogenic fungi, has been demonstrated on *Rhizoctonia solani* [33], *F. oxysporum* [34], *Gaeumannomyces graminis* [35, 36] and *B. cinerea* [37]. Other studies have shown that *Trichoderma* isolates can inhibit the growth of, and kill, wood decay fungi, such as *Lentinus lepideus*, by releasing VOCs [38]. However, although 6-PAP was the main volatile compound produced by *T. viride* in this study, this strain showed only slight antifungal activity, paticularly on *B. cinerea* and *M. miehei*. It is possible that the low activity shown by *T. viride* in this study was due to the culture conditions, for example, harvest time, substrate composition, culture temperature and light conditions, which were not optimised to each strain. Furthermore, it has been reported that volatile compounds emitted by *Trichoderma* sp., and their bioactivity, depend strongly upon the species and the substrate composition [39].

Some of the compounds identified in this study have been commonly reported in plants, for example, β-bisabolol and spathulenol [40,41]. Others compounds such as 2-methyl-1-butanol and 3-methyl-1-butanol [2,42] are typical volatiles emitted by fungal species, and have been suggested for use as chemical markers [43–45], e.g. 3-methyl-1-butanol, 2-hexanone and 2-heptanone, in detecting fungal growth in buildings [46]. Both isolates of *S. commune* produced high amounts of ethanol in relation to the others compounds. The production of this compound by microorganisms has been widely studied because of the production of potable, chemical and fuel ethanol, also known as bioethanol [47]. It has been detected in the headspace of mycelial cultures of other fungi, such as *Gliocladium roseum*, *Trichoderma* sp., *Mucor* sp. and *Penicillium expansum* [6,39,48]. However, in this study, ethanol was not detected in either *T. virens* or *Tr. versicolor* strains. Some of the compounds reported in this study have been shown to be constituents of the essential oils of the wood-rotting fungi *S. commune*, *Datronia mollis* and *Pycnoporus cinnabarinus* [49]. α-Curcumene, β-bisabolene and β-bisabolol have been identified in *S. commune* (Shi-2), and are constituents of the essential oil of *S. commune* [49]. Cadinene was present in the volatiles released from *Tr. versicolor* and was also identified in the essential oil of *S. commune* [49]. Bisabolol was the more abundant compound in the volatiles released from *S. commune* (Shi-2). Interestingly, this monocyclic sesquiterpene alcohol has been suggested for possible medical uses as a means of improving antioxidant capability and restoring the redox balance by antagonising oxidative stress [50]. Moreover, it has been suggested that bisabolol, and compounds of the same type, might enhance bacterial permeability and susceptibility to exogenous antimicrobial compounds [51], whereas bisabolene has been shown to have bactericidal and bacteriostatic effects against *Staphylococcus aureus* [52]. Both β-bisabolol and β-bisabolene were present in the two strains of *S. commune*. β-Bisabolol was the most abundant compound released from the more active strain, *S. commune* (Shi-2) (35.67%), and the percentage of β-bisabolene was similar (6.96 and 6.36%) in both strain of *S. commune*. These results, and those from the literature, lead us to propose that β-bisabolol might facilitate the antimicrobial activity of β-bisabolene. However, the possible toxic effects of high concentrations of ethanol [56.19 and 25.38% in *S. commune* (Shi-1) and *S. commune* (Shi-2), respectively] cannot be ruled out. Some studies have shown that CO_2 concentrations $>10\%$ can inhibit both the growth and sporulation of different fungal species [30]. This parameter was not evaluated in this study, and it is therefore not possible to rule out a possible negative effect of this compound on the growth of the phytopathogenic strains.

In summary, VOCs of both strains of *S. commune* were the most active in inhibiting *B. cinerea* growth. Future studies will be carried out to evaluate the effect of the composition of the growth medium on the production and bioactivity of VOCs, the dynamics of the volatiles released, and the antifungal effect of both individual and mixtures of identified volatile compounds in order to determine which volatile compounds are responsible for the biological activity of *S. commune*.

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